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REVIEW OF VACCINIA VIRUS AND BACULOVIRUS VIABILITY VERSUS VIRUCIDES

Patricia E. Buckley
Kevin P. O'Connell

RESEARCH AND TECHNOLOGY DIRECTORATE

Gary D. Ouellette

SCIENCE APPLICATIONS
INTERNATIONAL CORPORATION
Abingdon, MD 21009-1201

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There is a current need in the biological defense research, development, testing, and evaluation (RDTE) community for non-pathogenic viruses that can serve as simulants for poxviruses during phases of the development of new biological defense technologies. The need is particularly pressing in the field of decontamination sciences, where poxvirus simulants are essential for the RDTE of new virucidal treatments for equipment decontamination. This review summarizes what is known about the susceptibility to virucides of two candidate viral simulants, the baculoviruses *Cydia pomonella* granulovirus and *Heliothis zea* nuclear polyhedrosis virus and also compares this to similar information published for Vaccinia virus (VACV). In general, poxviruses and baculoviruses share similar susceptibilities to heat, extremes of pH, formaldehyde, sodium hypochlorite, germicidal UV radiation, and the effects of insect metabolism. The information reviewed supports the incorporation of baculoviruses into a viral simulant program; however, additional research is required to provide comprehensive comparisons between VACV and the candidate baculoviruses.

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PREFACE

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REVIEW OF VACCINIA VIRUS AND BACULOVIRUS VIABILITY VERSUS VIRUCIDES

1. INTRODUCTION

The development of a new viral simulant to supplement the use of bacteriophage MS2 in the research, development, testing and evaluation of new biological defense technologies is a focus of current research supported by the Joint Science and Technology Office (JSTO), Defense Threat Reduction Agency (DTRA). While MS2 has been used for decades as a simulant for viral human pathogens in biodefense research, it lacks structural, chemical and biological similarity to the orthopoxviruses (a family whose members include *Variola* virus (VARV), the causal agent of the disease smallpox), which is a viral threat agent of major concern and new focus of the biodefense community. Some of these dissimilarities include genome composition (single-stranded RNA versus double-stranded DNA), genome size (approximately 3500 bases versus 180,000 base pairs), and overall virion size (MS2 is approximately a 26 nm polyhedron, whereas variola virus is approximately 200 nm by 400 nm).

Ideally, a simulant for variola or other poxviruses would resemble poxviruses in at least gross physical composition and biochemical features, while possessing none of the traits (pathogenicity, ability to grow in mammalian hosts) that make orthopoxviruses genuinely or potentially dangerous to humans or other animals. Furthermore, the safety of an ideal viral simulant would be well documented, the simulant would be easy to obtain or produce and store, and would be accompanied by a suite of reagents and protocols that would empower researchers and engineers with its use. Fortunately, a class of viruses exists that meets many of these criteria.

Members of the Baculoviridae are pathogens of insect pests. Baculoviruses have been used for decades as part of "natural" integrated pest management (IPM) schemes for the control of such pests. Their safety has been documented in an extensive literature, and several baculoviruses have been approved for use by the U. S. Environmental Protection Agency (EPA). Baculoviruses, furthermore, resemble poxviruses in many of the ways described above: their virions tend to have particle sizes similar to poxviruses, their genomes are composed of double stranded DNA and are also of comparable size. As implied above, there are no known mammalian hosts of baculoviruses, and in some cases, their molecular biology has been described in great detail. We have identified two baculoviruses as candidates for new viral simulants: *Cydia pomonella* granulovirus (CpGV) and *Helicoverpa zea* nuclear polyhedrosis virus (HzSNPV). These viruses possess the attractive characteristics for use as a viral simulant set forth above; in particular, both are currently registered with the EPA. The form of each virus that is used as a pest control agent is commercially available in bulk. The baculoviridae are described in more detail below.

A primary goal of biodefense research is to develop simulants for poxviruses that reflect their sensitivity to decontamination agents and methods. The research agenda aims to incorporate these baculoviruses into the study of decontamination science and to produce datasets, which allow results obtained from working with baculoviruses to be laterally transferred into predicted results from RDTE work on the decontamination of poxviruses.

Specifically, research is under way to develop methods to culture and determine baculovirus survival, viability, and persistence. Overall, virus viability and persistence can vary greatly between environment, virus type, and virucidal agent. For example, in 1759, an exhumed coffin of an unknown smallpox victim buried 30 years previous still contained viable virus and was directly responsible for a smallpox outbreak in Somerset, after accidentally being opened (Ambrose, 2005). In contrast, Vaccinia virus (VACV), a similar orthopoxvirus, prepared in suspension will lose viability after 15 hr exposed at 50 °C (Woodroofe, 1960). Hence, understanding basic viral properties and the extent to which viruses lose viability in response to environmental stressors or virucidal substances and treatments is an integral component to viral simulant research and the biodefense community.

Viral persistence and viability outside the living host is dependent on the interaction with the environment. Yet, fundamental knowledge on the role virucidal effects, including the abiotic portion of the environment, is not collectively understood for CpGV and HzSNPV. The objective of this review article is to compare and assess the current state of understanding of the relative hardiness of VACV (poxviruses), and baculoviruses after exposure to various treatments, chemicals, or environmental conditions. We summarize here the susceptibility of baculoviruses to virucidal substances and conditions and relate this information to similar work published for VACV. We excerpt and summarize data for viability and persistence for these viruses exposed to various conditions and environments influencing virus survival and persistence.

2. TAXONOMY AND BIOLOGY

2.1 Baculovirus Overview.

Baculoviruses (Family: Baculoviridae) are double stranded DNA viruses characterized by a circular DNA genome and the presence of a large occlusion body or protein matrix (Rashidan et al., 2004). Baculoviruses are primarily pathogenic to arthropods and are distributed worldwide. Baculovirus-based insecticides are highly specific to the larval stage of their hosts and safe to humans and the environment. These characteristics make them particularly attractive as pest control agents (Inceoglu et al., 2001). The current taxonomy divides the family into two distinct genera based upon structural properties: Granulovirus (GV) and Nuclear Polyhedrosis Virus (NPV) (Rashidan et al., 2004). Baculoviruses are identified as potential candidates for viral simulant research because they physically resemble pox viruses with similar virion particle sizes (50-100 nm x 400 nm) and double-stranded DNA genomes.

2.1.1 CpGV.

CpGV is a granulovirus described from coddling moth larvae (*Cydia pomonella* L.) collected in Mexican apple and pear orchards (Tanada, 1964). The viral occlusions are formed in the nucleus of the cell with the capsule size averaging 393 x 207 µm (Tanada, 1964). Each granule contains only one virion (Jacques, 1977) and the occlusion matrix protein is comprised largely of granulin (Summers and Egawa, 1973). The genome is double-stranded DNA composed of 123.5 kilobase pairs (Luque et al., 2001). Over the past two decades, CpGV has been adopted for use in agricultural systems as an agent highly pathogenic to the coddling moth yet harmless to non-target organisms (Stará and Kocourek, 2003). It is currently available commercially in Europe and North America under several brand names, Cyd-X (Certis USA, L.LC.), Virosoft (BioTEPP Inc.), Carpovirosine (Sumitomo Corp.), Madex (Andermatt Biocontrol), and Granupom (Hoerst) (Lacey and Thomson, 2004).

2.1.2 HzSNPV.

The HzSNPV is a nucleopolyhedrovirus (NPV) highly effective as a biocontrol agent against the corn earworm, *Helicoverpa zea* (Boddie). Like other NPVs, the virus forms multi-sided occlusion bodies composed of a matrix protein known as polyhedron. The viral occlusions are often referred to as polyhedral inclusion bodies (PIB) and contain randomly occluded viral particles (Jacques, 1977; Tanada and Kaya, 1993). Physically, the baculovirus possesses a circular, double-stranded DNA genome comprised of 120 kilobase pairs [mol. Wt. of about 78 x 10^6] (Knell and Summers, 1984). HzSNPV has been widely used as a microbial insecticide since the mid 1970's as a result of its specificity towards the corn earworm and biological safety to humans and non-target organisms (Burges, et al., 1980). It is currently registered in the USA and sold under the trade name Gemstar (Certis USA, L.L.C.).

2.2 Poxvirus.

The poxviruses (i.e., *Poxviridae*) are large enveloped DNA viruses, which includes the causal agent to smallpox, VARV. In 1980, the World Health Organization (WHO), after a comprehensive vaccination program, announced the eradication of smallpox. This program utilized live VACV, a similar virus from the same genus, *Orthopoxvirus*. A VACV infection is mild and typically asymptomatic in healthy individuals. Immune response generated from a VACV infection protects the person against a lethal smallpox infection. Because of this characteristic, VACV was primarily employed for vaccination against smallpox via inoculation (Henderson et al., 1999). VACV is comprised of a linear, double-stranded DNA genome approximately 190 kb in length (McClain, 1997), a size comparable to VARV and the baculoviruses. Because of these close physical and chemical similarities with VARV, VACV is typically employed as a surrogate virus for smallpox research. Due to a paucity of information on VARV, this review identifies results from previous VACV studies to demonstrate how VARV would hypothetically respond under similar study conditions.

3. OVERVIEW OF FACTORS INFLUENCING VIRUS VIABILITY

Numerous physical, chemical and biological factors influence virus persistence and activity. Some of the primary physical factors involved in the persistence of viruses outside living hosts include temperature, UV light, moisture (or relative humidity) and pH. These factors may interact with each other, and their effect can be additionally altered by virucidal chemicals, formulation additives, and a variety of substrates, including soil, fabrics and plant foliage.

Overall, studies on virus hardiness and persistence are quite different for VACV and baculoviruses. Research on VACV has focused mainly in three areas, (1) virus persistence, (2) potency of stored vaccine preparations as well as, (3) disinfection measures and virucidal materials to interrupt the chain of infection for the prevention of disease spread in the Healthcare and Food industry (Sattar et al., 2000). Comparatively, baculovirus studies are agricultural based with direct focus on improving Bio-insecticide performance (Falcon, 1969). Research has targeted physical and biotic factors of viruses such as virulence; virus stability and persistence in field applications; virus dispersal; and viral transmission. Among physical factors of the environment, baculovirus studies have focused on the effects of sunlight, temperature, pH and moisture as well as the use of additives to prolong the hardiness of a virus.

3.1 Ultraviolet Radiation.

The literature concerning the responses of viruses to UV radiation largely concerns baculoviruses (Ignoffo, 1992). However, UV radiation is a destructive abiotic factor affecting the persistence and viability of many viruses including poxviruses (Sobsey and Meschke, 2003). Over the years, a considerable variety of approaches have been undertaken in the study of the extent to which UV radiation can adversely affect viruses and have included natural sunlight; monochromators; and, germicidal lamps. Table 1 summarizes the effect of UV radiation on virus persistence.

3.1.1 VACV.

The extent to which viral pathogens of humans persist in the environment to reach other hosts is of primary public health interest and concern. Hence, numerous studies have focused on germicidal UV cell (UVC) light (254-nm) for airborne VACV inactivation and the control of microbial infection spread (Jensen, 1964; McDevitt et al., 2007; Rauth, 1965). As evidenced from studies, VACV is readily inactivated by UV light (Henderson et al., 1999). VACV released as an aerosol, devoid of UV light, is capable of survival up to 24 hr (Henderson et al., 1999). In an earlier study, Jensen (1964) reported VACV is highly susceptible to UVC with >99% viral inactivation with short exposure times. McDevitt et al. (2007) found similar results, demonstrating VACV susceptibility to UVC. In 1965, Rauth investigated the UVC sensitivity in the range 225–302 nm for 12 medically important viruses including VACV, and postulated the susceptibility of vaccinia to UV inactivation is the result of its relatively large size and adsorption cross-section compared with those other viruses (Rauth, 1965). In a

comparison of UV spectra effects on VACV, Sime and Bedson (1973) observed greater deactivation in the range between 280-300 nm. These results are comparative to those found with baculoviruses.

The stability of viruses exposed to ionizing radiations has also received attention. In 1939, Gowan and Lucas reported an exponential loss of VACV activity following exposure to x-rays (McCrea et al., 1960). A study by McCrea et al. (1960) investigated VACV inactivation using low-voltage electrons. The research revealed doses of energy (1000 V) penetrating the virus as far as 330 A only resulted in slight virus inactivation. However, as the energy was increased to 1500 volts, with electron penetration occurring between 500 and 700 A, a rapid loss of virus activity was observed.

3.1.2 Baculovirus.

Numerous studies have investigated effects of UV light inactivation of baculoviruses (Bullock et al., 1970; Glen and Payne, 1984; Griego et al., 1985; Kienzle et al., 2003; Morris, 1971). Generally, the virucidal effect of UV radiation is rapid and has been partly attributed to the adverse affects on DNA or virus-associated structural proteins (Hunter-Fujita et al., 1998; Ignoffo and Garcia, 1994).

In the field, baculovirus activity can be completely lost in <24 hr, but the mean half-life varies from 2 to 5 days (Jaques, 1985; Moscardi, 1999). One field study suggested CpGV half life, under natural light, ranges between 48 to 72 hr (Kienzle et al., 2003). Similar findings were observed by Jaques et al. (1987). A field application study by Glen and Payne (1984) showed CpGV virus activity was reduced in half after 3 days' exposure to natural light with some activity persisting up to 4-8 weeks. Bullock (1967) reported HzSNPV retained little activity 2 days after application on cotton. Similar findings on cotton were reported by Ignoffo and Batzer (1971) and Yearian and Young (1974), on soybean foliage by Ignoffo et al. (1974), and on corn silk by Ignoffo et al. (1973).

Studies have suggested field applications of baculoviruses are not completely inactivated at a constant rate. Huber and Luedcke observed a bi-segmented inactivation curve for CpGV; 99% initially inactivated with a small proportion persisting much longer (Kienzle et al., 2003). Those results are very much in line with an earlier study by David et al. (1968). After a 3-hr exposure to natural sunlight, a 0.05% suspension of *Pieris brassicae* Granulovirus (PbGV) rapidly inactivated on cabbage leaves with complete inactivation after 12-19 hr (David et al. (1968).

The inactivation of baculoviruses under UV lamps and simulated sunlight is more rapid than demonstrated by direct sunlight. Ignoffo et al. (1977) estimated a half life of 2 hr for *Pieris rapae* Granulovirus (PrGV) and *Helicoverpa spp.* NPV exposed to UV lamps. In support of these findings, Ignoffo et al. (1989) estimated that the half lives of occluded and non-occluded HzSNPV virions were 5.8 and 6.3 hr, respectively, under simulated sunlight (320-380 nm). In other studies, Ignoffo and Batzer (1971)

showed deposits of HzSNPV on cotton leaves and glass plates were readily inactivated by 4-hr exposures to either artificial (range 215-260 nm and 290-400 nm) and/or natural light resulting in *ca* 70% of virus being inactivated within the first 4 hr of exposure. In a study by Gudauskas and Canerday (1968), virus stock suspension from *Trichoplusia ni* Nuclear Polyhedrosis Virus (TnNPV) and HzSNPV were exposed to UV light (254 nm) emitted from a Spectroline Lamp for varying times and distances. The TnNPV was found more stable with significant-to-complete virus inactivation by exposures at 2-6 in. between 4-10 min; where as, similar inactivation for HzSNPV was reported at 2-8 in. for 1-5 min. In a recent study, Roberts and Hope (2003) investigated the effects of high intensity broad spectrum pulsed light from a PureBright® system on virus inactivation. PureBright® uses wavelengths ranging from 200-1100 nm and short flashes (300 µS) at an intensity 1000 times that of conventional light. Roberts and Hope (2003) reported VACV completely inactivated at total fluences >1.5 (J/cm^2).

The subject of UV inactivation cannot be left without considering the possibility of interactions with other abiotic factors attributing to variations in persistence. Using simulated sunlight, Ignoffo and Garcia (1992) studied the interaction of UVA and UVB (290-400 nm) with pH (at 3, 6 and 9), water, and temperature (at 10, 22, 35 and 50°C) on the inactivation of HzSNPV. No interactions were observed with either temperature or pH; however HzSNPV was more sensitive when exposed to water than dry virus. McLeod et al., (1977) reported a significant interaction between the effects of temperature and UV radiation on virus inactivation. Inactivation of HzSNPV exposed to sunlight at 45 °C was extensive in comparison to virus exposed at 30 °C.

There is considerable evidence that the UV portion of sunlight inactivates viruses, specifically, UVB (280-320 nm) and UVC (185-280 nm) because wavelengths in the range from 200 to 280 nm are highly absorbed by nucleic acids (Arthurs and Lacey, 2004; Bullock et al., 1970; Jaques, 1985; Sime and Bedson, 1973; Sobsey and Meschke, 2003). Since wavelengths shorter than 290 nm do not penetrate to the earth's surface, virus inactivation in the field are due to wavelengths >290 nm (Griego et al., 1985). Bullock et al. (1970) reported that 2-hr exposure to wavelengths of 257 and 307.5 nm significantly inactivated *Helicoverpa spp.* NPV virus while exposure to a wavelength of 364 nm and a broad-band mixture of visible and infrared light did not affect activity in bioassays. In a related study by Griego et al. (1985), monochromatic UV radiation at wavelengths 290, 300, 310 and 320 nm inactivated *Orgyia pseudotsugata* Nuclear Polyhedrosis Virus (OpNPV). Similarly, David (1969) observed virus inactivation decreased as the UV wavelengths progressively increased within the range 250-330 nm, with the greatest inactivation found between 250 and 270 nm for PbGV. UV radiation exposure at 366 nm for periods up to 100 hr was demonstrated to be only slightly virucidal to *Lambdina fiscellaria* Nuclear Polyhedrosis Virus (LfNPV) (Morris, 1971).

Studies have demonstrated that deposits of impure viral preparations are not inactivated as quickly as pure preparations when exposed to UV or natural sunlight (David and Gardiner, 1966; David et al., 1971a; Griego et al., 1985; Jaques, 1977). It is suggested that dark color and protein content of crude suspensions prolong virus activity (Jaques, 1977). Griego et al. (1985) observed crude virus suspensions preserve virus better than highly purified inclusion bodies (Griego et al., 1985). These findings agree with David (1969), who observed crude preparations of PbNPV remained active after 120-hr exposure to UV light (253 nm) as dry film. Similarly, David et al. (1971a) reported viral activity of PbGV persisted for 7 days, while held in darkness, with the incorporation of hemolymph from host insects. Such discoveries led to the development and incorporation of sun protectants into baculovirus formulations as reviewed below.

3.2 Temperature.

The deleterious affects of temperature on virus persistence is significant in storage and in the environment, but is comparably less important than solar radiation (Jacques, 1985). Inactivation by heat is a nonselective process and affects various functions associated with the virus. Virucidal effects of temperature operate through several mechanisms, including protein denaturation, RNA damage, and influence on microbial or enzymatic activity (Harper et al., 1978; Sobsey and Meschke, 2003). Storage data for VACV and baculoviruses have been collected under a variety of conditions with viruses stored in suspensions, as PIBs, as dried powders, at room temperature, refrigerated, and in some cases frozen. Generally, studies have shown as temperatures are lowered, virus inactivation is greatly prolonged. Table 2 summaries the effect of temperature on viral storage.

3.2.1 VACV.

The thermostability of VACV was a major concern for the development of efficient vaccination programs (Henderson et al., 1999). In general, poxviruses show high environmental stability compared with many other viruses (Rheinbaben et al., 2006; Wolf and Croon, 1968). VACV stored at low temperatures have been noted to resist deterioration for considerable periods of time. It has been reported that VACV has retained virulence for periods exceeding 15 years frozen in buffer at -20 °C (Rheinbaben et al., 2006). Alternatively, while dried, VACV can be stored for >35 weeks at 4 °C without any loss in viability (Rheinbaben et al., 2006). However, dried VACV embedded in rabbit dermal scabs inactivated after a 1-hr incubation at 90 °C in saline solution (Schümann and Grossgebauer, 1977).

When aerosolized, VACV has been demonstrated much less persistent. Aerosolized VACV was almost completely inactivated within 6 hr at 31-33 °C and 80% humidity (Henderson et al., 1999). As the temperature decreases to 10-11 °C and humidity is decreased to 20%, the survival rate of virions increases; after 24 hr, only 33% of the virus was reported inactivated (Henderson et al., 1999). When frozen in buffer at -20 °C, a titer reduction of only 3 log-steps is reported within 15 years

(Rheinbaben et al., 2006). Freeze-dried material persisted at 45 °C for a period of 6 years with little signs of inactivation (Fenner et al., 1988).

It is evident that temperatures above 50 °C rapidly inactivate viruses within a few hours. Kaplan (1958) investigated the persistence of VACV and determined inactivation curves at temperature ranges between 50-60 °C. The results from this study demonstrated a rapid fall of infectivity followed by a complete inactivation at a much slower rate. Remington et al. (2004) inactivated VACV by pasteurization at 60 °C in a variety of protein solutions. Approximately a 2 $\log_{10}$ inactivation of VACV was observed after 1-hr pasteurization in alpha$_1$-proteinase inhibitor solution; within 3 hr of exposure, no active VACV was detectable. Similarly, in human plasma protein solution, VACV was reduced by 4 $\log_{10}$ within 30 min; and after 2 hr, virus activity was undetectable.

Earlier work demonstrated the effects of metal ions on heat inactivation of VACV. Wallis et al. (1962) reported the stability of VACV suspensions was enhanced by the addition of 2M-Na^+; virus was protected up to 4 hr at 50 °C and 24 hr at 37 °C. In support of these findings, Kaplan (1963) reported the reaction velocity of inactivation in suspensions of VACV decreased as the concentration of Na_2HPO_4 increased. Further investigating revealed in the presence of metal ions, specifically a mixture of 100 mM-Na^+ and 1 mM-Mg^{2+}, VACV was significantly more stable at 55 and 60 °C when compared with suspensions without ions.

3.2.2 Baculovirus.

There appears to be much variation in the stability of baculoviruses where storage temperature is concerned. In general, baculoviruses are quite stable at normal room temperatures. Temperatures below 10 °C increase the persistence of HzSNPV (Ignoffo, 1992). Stored at 5 °C, HzSNPV inclusions were still active after 25 years storage (Ignoffo, 1992). Shapiro and Ignoffo (1969) reported HzSNPV in water suspension was still present after 120 and 225 days storage at 5, 37, and 50 °C, while a 10-min exposure at 80 °C completely inactivated HzSNPV. The study concluded that the virus should still be active after 500 days storage at 5 °C (Shapiro and Ignoffo, 1969). Alternatively, David et al. (1971b) demonstrated that purified *Pieris brassicae* GV, in a dry form, lost a significant amount of activity after 2 days at 20 °C.

Research has generally indicated that baculoviruses rapidly inactivate at temperatures above 60 °C but withstand maximum temperatures normally encountered in field environments for short periods (Jaques, 1977). Studies investigating the effects of temperature on baculovirus viability have focused largely on ambient temperature ranges experienced during growing seasons (Ignoffo, 1992; Jaques, 1977). At temperatures approaching 50 °C, the persistence of HzSNPV significantly decreased to <100 days (Ignoffo, 1992). Gudauskas and Canerday (1968) reported TnNPV was inactivated by approximately 70% at 80 °C and 97% at 82 °C after heating for 10 min. Comparatively, HzSNPV was inactivated by approximately 40 and 100% at 75 and

80 °C, respectively. Both viruses were totally inactivated at 88 °C after 10 min. Studies have shown *Heliothis virescens* NPV withstanding temperatures of 60 °C for 2 hr and still remaining active at 93.3 °C for 30 min (Stuermer and Bullock, 1968).

As mentioned above, the pernicious affects of temperatures target various functions of viruses. Temperature has been observed to inhibit replication in some baculoviruses, such as *Anticarsa gemmatalis* Nuclear Polyhedrosis Virus (AgNPV) (10 °C and 40 °C), TnNPV (39 °C), and PrGV (36 °C) (Johnson et al., 1982; Moscardi, 1999). One explanation is that a similarity between the virus and insect host development temperature is expected; as temperatures rise higher than normally associated with the virus-insect relationship, virus replication becomes adversely affected. Also in this regard, studies have suggested, temperature affects the integrity of viral proteins; specifically, higher temperatures breakdown and degrade proteins. The effect of temperature on inclusion body protein was observed to play an important role for the stability of baculoviruses in storage (Jaques, 1977).

3.3 Moisture and Relative Humidity.

The effect of moisture and relative humidity (RH) on virus persistence in the environment chiefly varies with virus type. As one would expect, viruses with higher lipid content tend to be more viable and persistent at a lower RH (Sobsey and Meschke, 2003). Both VACV and baculoviruses are generally sensitive to conditions of moisture and RH; the stability of these viruses is often improved through desiccation (David et al., 1971a).

3.3.1 VACV.

Studies have demonstrated VACV is relatively stable in dry environs, but when held in a high humidity atmosphere, it rapidly inactivates (Sidwell et al., 1966). In scabs, RH was shown to be an important factor limiting the viability of VARV (Huq, 1976; MacCallum and McDonald, 1957). At ambient room temperature and RH between 85% and 90%, no viable virus was detected after 8 weeks. When the RH was lowered (<10%), the viability was prolonged to 12 weeks. Similarly, Sidwell et al. (1966) reported VACV was less stable in high humidity environments (78% RH). In line with these findings, McDevitt et al. (2007) showed the survival of aerosolized vaccinia in the presence of UVC is significantly influenced by RH.

3.3.2 Baculovirus.

Surface moisture may influence the susceptibility of baculovirus deposits to inactivation by UV light. Consistent with the studies on VACV, David (1969) observed PbGV inactivated more rapidly in wet virus films exposed to UV radiation than in dry films, as Jaques (1967) reported for TnNPV and, Ignoffo and Garcia (1992) for HzSNPV. These findings suggest that surface moisture favors the inactivation of baculoviruses exposed to UV radiation.

Surprisingly, there exists little research on the effects of rainfall on baculovirus persistence. Ignoffo et al. (1965) reported that heavy rain produced little effect on HzSNPV. Similar reports have been made for PbGV and *Smithiavirus pityocampae* (Burgejon and Grison, 1965; David and Gardner, 1966). Generally, rainfall is considered an important dispersal mechanism for NPV in the habitat (Bird, 1961; Jacques, 1964; Young, 1990). In a study by Young (1990), virus inclusion bodies from cadavers of *Psedoplusia includens* (Walker) and *Anticarsa gemmatalis* (Hübner) were easily washed and dispersed from soybean foliage by sprinkler irrigation. Bird (1961) reported rain as a primary agent responsible for spreading sawfly baculoviruses throughout trees. Alternatively, Ignoffo et al. (1997) observed that HzSNPV did not wash from soybean leaves after simulated rain (1.25 cm in 30 s). Similar results were reported for HzSNPV on cotton leaves after a natural rain (Bullock, 1967) and PbGV exposed to either simulated rainfall or detergent-water rinse (David and Gardiner, 1966).

3.4 pH.

The stability of viruses either in suspension, aerosols or on surfaces can also be adversely affected by pH. In general, the inclusion body protein or envelope in which baculovirus and VACV are embedded provides the virus particle with some degree of protection against the deleterious effects of chemicals. Pox and baculoviruses are stable at pH near neutrality, and, from pH 4 to 8, these viruses have good short-term persistence (Andrews and Sikorowski, 1973; Ignoffo and Garcia, 1966; Rheinbaben et al., 2006; Young and Yearian, 1976).

3.4.1 VACV.

One physical characteristic of Poxviruses is the possession of low lipid content (Rheinbaben et al., 2006). As a result, poxviruses tend to be less sensitive to the extreme pH ranges found in organic solvents and disinfectants compared with other enveloped viruses. Beard et al. (1938) reported VACV is most stable between the range of pH 4.5 and pH 10 with immediate virus inactivation below pH 2.5 or above pH 11.5.

3.4.2 Baculovirus.

The virucidal effects of pH have been studied more extensively for baculoviruses. Ignoffo and Garcia (1966) observed a significant reduction in virus activity for inclusion bodies exposed at pH 1.2 and pH 12.4. Similar results were reported for HzSNPV at pH 2 and pH 12 (Gudauskas and Canerday, 1968). Virus inactivation was recorded at 50 and 88% after 30 min and 24 hr, respectively, and suspended at pH 2 in 1.0 M phosphate buffer. Comparatively, virus suspended at pH 12, in 1.0 M phosphate buffer, was inactivated by 40 and 92% after 30 min and 24 hr, respectively. In a study by Thomas et al. (1973), the effects of soil pH on virus persistence of TnNPV was investigated and observed that at a lower pH (4.83 – 5.22), the virus rapidly inactivated over a month (Tomas et al., 1973).

The effects of alkalis on baculoviruses are important for virus stability in the field habitat. Strongly alkaline pH (>10.0) is reported to dissolve the inclusion body protein of baculoviruses, releasing the virion (Kawarakata et al., 1980; Shapiro and Ignoffo, 1969). Kawarakata et al. (1980) observed prolonged (10-120 min.) exposure of *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV) to an alkaline solution results in the disruption of the virus envelope, subsequently releasing the virion. Similarly, studies have shown HzSNPV inactivation in solutions of pH 9.0 or above, with rapid inactivation occurring as the pH approaches 10.0 (Andrews and Sikorowski, 1973; Ignoffo and Garcia, 1966; Young and Yearian, 1976). Slightly alkaline conditions (pH 7.8) have been employed to liberate virions in *Trichoplusia ni* Granulovirus (TnGV) (Summers and Paschke, 1970). The alkaline pH (8.0 - 10.0) on cotton leaf surfaces is reported to inactivate HzSNPV (Young and Yearian, 1976). A study by Andrews and Sikorowski (1973) showed HzSNPV inactivates in dew from cotton leaves whose pH ranged between 8.2 and 9.1. Young and Yearian (1974) observed that HzSNPV inactivated more rapidly on cotton leaves than on soybean or tomato whose leaf surfaces are close to pH 7.0 (cited in Young and Yearian, 1976). In support of these findings, exposure to pH 9.3 cotton dew resulted in a substantial loss of viral activity (McLeod et al., 1977). However, Young and Yearian, (1974) observed no significant difference between the three hosts when cotton leaves were protected from the sun (Yearian and Young, 1974) suggesting an interaction with UV radiation. One study reported improved virus persistence when the virus was buffered to a neutral pH (Falcon, 1969). In contrast, Young and Yearian (1976) found virus in a neutral phosphate buffer failed to increase persistence on cotton leaves.

4. ENVIRONMENTAL AND SUBSTRATE INFLUENCES ON BACULOVIRUS AND VACV SURVIVAL

4.1 Soil.

The stability and persistence of baculoviruses in the soil differ substantially partly because of exposure of viruses to different environmental conditions. Overall, there are few studies on the stability of viruses in the soil. Tanada and Omi (1974) reported GV and NPV viruses from several insects persisting in soil up to several months, even during winter months. In a similar study, Jaques (1964) reported TnNPV persisting in soil for periods exceeding a year.

As expected, the pH of soil plays a vital role in viral inactivation in soil. Thomas et al. (1973) investigated the effects of soil pH on virus persistence of TnNPV. The study tested a range of soil pH (4.83-7.17) over three month intervals and demonstrated that at a lower pH (4.83, 5.02, and 5.22), the virus rapidly inactivated (Tomas et al., 1973). After 9 months, viruses exposed to lower pH soils were mostly inactivated. In contrast, virus in soils closer to pH 7persisted up to 12 months; though there was a significant decrease in recovery of active virus (Tomas et al., 1973).

4.2 Phyllosphere.

Persistence of viruses on foliage is influenced primarily by their exposure to sunlight. Field studies on the impact of UV radiation have focused on the estimation of physical loss of baculovirus occlusion body deposits over time from the test plant surfaces. As discussed previously, virus inactivation by UV radiation is regarded as the most important abiotic factor affecting baculovirus stability. On plants, the effect of sunlight is greatly influenced by two main factors, (1) crop architecture, and (2) the position of virus on the plant. Studies have found HzSNPV on the adaxial surface of cotton leaves in the field have become inactive after three days (Bullock, 1967). Ignoffo et al. (1973) assessed the stability of HzSNPV on corn silks and reported virus activation up to 24 days, though significantly less activity than reported for activated carbon stabilized virus. A Combination of UV light and the alkaline pH of cotton leaf surfaces have been reported to inactivate HzSNPV (Yearian and Young, 1974; Young and Yearian, 1976).

The decline in activity of baculovirus deposits on trees is typically rapid, but the rate of inactivation is usually slower than on agricultural crops. In pine trees, NPV on the lower canopy position persisted significantly longer as a result of decreased UV exposure (Killick and Warden, 1991). Comparably, Kaupp (1983) suggested *Neodiprion sertifer* Nuclear Polyhedrosis Virus (NsNPV) can persist for periods up to 2 years on pine foliage. A thorough search of literature revealed no studies on the survival or persistence of poxviruses on plant materials.

4.3 Textiles.

Research involving virus persistence on fabrics have received much attention over the years (Sidwell et al., 1966, 1969); specifically the considerable importance of the potential dissemination of viruses by various fomites such as clothing and other textiles. Sidwell et al. (1966) investigated the persistence of VACV on a variety of cotton and wool fabrics and reported vaccinia persisted up to 14 weeks on wool fabrics with low humidity. VACV persisted for a shorter period on the cotton fabrics (Sidwell et al., 1966). In a similar study, MacCallum and McDonald (1957) reported VARV in scabs survived 3 to 4 months in raw cotton at 58% RH. To our knowledge, there have yet been studies made on the effect of textiles on baculovirus survival.

4.4 Effect of Insect Metabolism on Viruses.

Few studies have focused on the effect of viral inactivation by insect metabolism. One study investigated the infectivity of *Helicoverpa punctigera* NPV through the gut of a predator insect (Beckman, 1980). Active viral inculsion bodies were collected from the excreta of the predatory, *Nabis tasmanicus* Remame up to 4 days after with no loss of virus activity (Beckman, 1980). Similarly, Bartzokas et al. (1978) studied the effect of formaldehyde fumigation on VACV infected cockroaches to determine the limits of survivability. The study found VACV ingested before

formaldehyde fumigation remains viable inside the cockroaches gut and active virus may be excreted up to 5 days later.

5. VIRUCIDAL CHEMICALS

5.1 VACV.

The effects of virucidal chemicals on VACV inactivation has been studied for several decades. Research investigating chemical disinfectants on surfaces for the prevention of disease spread has focused mainly on the Healthcare and Food industry (Sattar et al., 2000). Discussed below are specific details, from studies, on the known or expected activities of major classes of chemical virucides against VACV.

5.1.1 Aldehydes.

Numerous studies have demonstrated formulations based on formaldehyde and glutaraldehyde as highly effective at inactivating VACV. In the interest of learning the effects of formaldehyde vapor disinfection on smallpox, a study by Grossgebauer et al. (1975) was conducted using VACV as a surrogate virus. VACV, embedded in scabs, was exposed to a recommended dose of formaldehyde vapor (5 g/m^3) for 6 hr at 75% RH in hospital rooms. Grossgebauer et al. (1975) reported up to 85% virus inactivation at the recommended dose; however, by doubling the dose, the virus inactivation increased up to 95%. In suspension assays, Amies (1960) observed complete VACV inactivation within 150 to 200 hr in tests combining 0.006 M formaldehyde, 0.02 M glycine and virus. Paraformaldehyde, in concentrations of 0.3, 1.5, or 7.5%, rapidly inactivated recombinant VACV in baby hamster kidney cells (Hulskotte et al., 1997). At concentrations of 1.5% or 7.5%, VACV inactivated in <10 min; however, at the lower concentration (0.3%), the virus persisted up to 50 min. Glutaraldehyde is known to have virucidal activity against VACV as well as against other viruses (Sidwell et al., 1970). Sidwell et al. (1970) reported laundering bed pads with glutaraldehyde, in combination with a detergent, inactivated VACV and lowered virus titers to below detectable levels. Bachrach and Rosenkovitch (1972) compared the effect of aldehydes on the inactivation of VACV by a plaque assay. Oxidized spermine was reported more affective than glutaraldehyde or formaldehyde at inactivating a VACV suspension at 37 °C for 10 hr. Oxidized spermine, at a concentration of 0.82 mM, completely inactivated the virus. Schümann and Grossgebauer (1977) reported complete inactivation of VACV embedded in rabbit dermal scabs with 90-min exposures to suspensions of either 2% glutaraldehyde or 2% formaldehyde.

5.1.2 Amphotericin B Methyl Ester.

Jordan and Seet (1978) studied the antiviral effects of Amphotericin B Methyl Ester (AME), an antimicrobial agent, on several viruses including VACV in a plaque reduction assay. The concentration of AME resulting in a 50% inactivation of the plaque forming units, after a 60-min exposure at 35 °C, was reported to be 5.0 µg/mL. Jordan and Seet (1978) suggested lipid components in the host cell membrane, which incorporate into the viral envelope, may serve as a susceptible site for AME.

5.1.3 Ascorbic Acid.

Early experiments on the viability of VACV in the presence of various reducing agents revealed ascorbic acid was strongly virucidal (Kligler and Bernkopf, 1937). Turner (1964) reported quantities of ascorbic acid, as little as 62.5 µg/mL, completely inactivated VACV exposed for 2 hr at 37 °C; under similar conditions, 100 µg/mL inactivated VACV in the span of 15 min. Moreover, in the presence of Cu^{2+} (5 µg/mL), a catalytic effect was observed; the rate at which VACV inactivated significantly increased when exposed to ascorbic acid.

5.1.4 Dithiothreitol Reducing Agent.

A study on the effects of bond reducing chemicals on viruses by Carver and Seto (1968) investigated the susceptibility of virus inactivation to dithiothreitol (DTT), a disulfide bond reducing agent. The DTT inactivated VACV after a 3-hr incubation at 37 °C. Carver and Seto (1968) postulated the lipoprotein envelope present around poxviruses accounted for VACV susceptibility to DTT.

5.1.5 Ethylene Oxide.

Many chemicals have been considered as virus inactivating agents for the sterilization of potential fomites. One agent, ethylene oxide, $(CH_2)_2O$, possesses antimicrobial properties against a suite of fungi, bacteria and viruses (Sidwell et al. 1969). Sidwell et al. (1969) investigated the virucidal effects of ethylene oxide gas, on VACV, applied using Steri-Vac sterilizer following two cycle regimes: 29 °C for 180 min and 60 °C for 48 min. Under both experimental conditions, Sidwell et al. (1969) reported VACV inactivation with virus titer reduced to less than detectable limits. Similar findings were observed by Klarenbeck and Tongeren (1954), and Hoff-Jørgensen and Lund (1972).

5.1.6 Quaternary Ammonium.

Another investigation reported fabrics impregnated with quaternary ammonium compounds expressed virucidal effects and significantly inactivated the VACV (Siwell & Dixon, 1969). In 1971, Oxford et al. studied the effects of a mixture of virucidal compounds comprised of quaternary ammonium (benzalkonium chloride),

a detergent (Triton X100) and citric acid on virus inactivation. Oxford et al. (1971) reported VACV inactivation after a 20-min incubation period.

5.1.7 Sanitary Alcohols.

In recognition of the need for broad spectrum hand disinfectants, new formulations of sanitary rubs have been tested for virucidal activity. Kampf et al. (2007) tested the effects of three common alcohol-based sanitary hand rubs against enveloped viruses including VACV. All three sanitary rubs contained at least 75% alcohol and were tested at exposure times of 15, 30, and 60 s. Kampf et al. (2007) reported significant viral inactivation (≥4 log 10 steps) after 15 s with infectivity reduced to below detectable levels. In an earlier study, Schümann and Grossgebauer (1977) observed complete disinfection of VACV from hands using 70% isopropyl-alcohol with a 2- to 5-min exposure. Similarly, VACV embedded in rabbit dermal scabs were completely inactivated after 3-hr exposures in suspensions of 60% n-propyl-alcohol, 70% isopropyl-alcohol, or 80% ethyl-alcohol (Schümann and Grossgebauer, 1977). Wutzler and Sauerbrei (2000) reported a formulation consisting of 0.2% paracetic acid (PAA) and 80% ethanol (v/v) completely inactivated VACV after a 30-s exposure. In addition, Kramer et al. (2006) studied the virucidal effects from an alcohol-based formulation including 55% ethanol (w/w) with 10% (w/w) propan-1-ol, 5.9% (w/w) propan-1.2-diol, 5.7% (w/w) butan-1.3-diol and 0.75% phosphoric acid. In suspension tests, VACV inactivated after a 30-s exposure.

5.1.8 Solvent/Detergent Treatment.

Treatments including combinations of solvents/detergents have been widely used in the healthcare industry to inactivate viruses within plasma or blood products. Reminton et al. (2004) investigated the effects of two solvent/detergent combinations on the inactivation of enveloped viruses. Cocktails of VACV (10% v/v) and Tri-(n-butyl)-phosphate (TNBP)/Tween 80 in solutions of anti-haemophilic factor incubated at 28 °C for 6 hr reduced virus titer by 3 $\log_{10}$. The observed resistance of VACV to TNBP/Tween 80 treatment was in support of earlier findings by Roberts (2000). Comparatively, combinations of VACV (10% v/v) and TNBP/Cholate in solutions of intravenous immunoglobulin completely inactivated the virus >1 hr (Remington et al., 2004). The healthcare industry also commonly includes treatments using caprylic acid salts in plasma products. Remington et al. (2004) reported suspensions of VACV (10% v/v) in Gamunex® intermediate solution containing 10% sodium caprylate resulted in complete virus inactivation within 3 min when incubated at 22 °C in 20 mM caprylate.

5.1.9 Surface Disinfectants.

The virucidal activity of monopercitric acid (MPCA) was determined by suspension tests against nonenveloped and enveloped viruses including VACV (Wutzler and Sauerbrei, 2004). The study showed that viruses were 99.9% inactivated by a 0.5% concentration within 30 s demonstrating MPCA as a suitable candidate for a

disinfectant. Sugimoto and Toyoshima (1979) reported on the inactivation of VACV by N^{α}-Cocoyl-L-Arginine Ethyl Ester, DL-Pyroglutamic Acid Salt after a 30-min exposure at room temperature. At all tested concentrations (0.025, 0.05, 0.1, and 0.25%), there was >90% inactivation of VACV.

Ferrier et al. (2004) studied the virucidal effects of a non-corrosive commercial disinfectant whose composition combines a suite of viral inactivating agents including quaternary ammonium, aldehydes, alcohol and detergent. The disinfectant Sanytex® was assessed in the presence of protein in suspension and surface tests against VACV. Suspension assays showed 1% concentrations of Sanytex effectively inactivating (log reduction >4) VACV in the presence of 3 mg/mL protein. In parallel, Ferrier et al. (2004) tested the virucidal activity of sodium hypochlorite in suspension assay and reported 0.525% concentration of active chlorine inactivating VACV (log reduction >4) with 10 mg/mL protein present. In surface tests, Sanytex was less effective requiring higher concentrations and longer exposure times for viral reduction. The virus was effectively inactivated after 15 min exposure by 10% detergent concentration and after 10 min by a 30% concentration of detergent containing 10 mg/mL protein concentration. Overall, the study demonstrated Sanytex as a highly effective disinfectant for use in laboratories and clinical facilities.

One study by Gaustad et al. (1974) evaluated the virucidal efficacy of three surface disinfectants (quaternary ammonium, phenolic, and iodophor) on a simulated hard-environmental surface with a 10-min exposure at 20 °C. Of the three disinfectants, the quaternary ammonium compound was the most effective at inactivating VACV (ca. 4.5 Log^{10} reduction); however, iodophor and phenolic produced similar results with an inactivation of 3.9 Log^{10} and 3.7 Log^{10}, respectively.

As illustrated above, various disinfectants are used for inactivation of pox viruses in laboratory, clinical and hospital facilities. However, these are generally not suitable for the home environment, posing both health and environmental hazards. One study targeted the effects of common household disinfectants on virus inactivation (Butcher and Ulaeto, 2005). Butcher and Ulaeto (2005) assessed common disinfectants on VACV and reported a household, chloroxylenol-based disinfectant completely inactivated VACV at ambient room temperature.

5.1.10 Miscellaneous.

With recent concerns over the use of smallpox virus as a biological weapon, broad pox-inhibitory agents have been investigated. ST-246 (4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide) was identified as a potential chemical agent for use against smallpox virus. In a study by Yang et al. (2005) several inhibitory properties for ST-246 were identified. In cell culture, ST-246 was demonstrated a specific inhibitor of poxvirus replication in two separate VACV assays: 1), in a cytopathic effect assay, an EC_{50} for inhibition was recorded at 0.01 µM, and; 2), a lack of extracellular virus formation in a virus yield assay after exposure to 5 µM ST-246. Similarly, plaque formation was completely inhibited in

cowpox virus exposed to ST-246. In orally administered ST-246 assays, formation of VACV induced tail lesions was inhibited in Mice infected with live VACV via tail vein. The series of assays conducted by Yang et al. (2005) clearly identified ST-246 as a broad virucidal chemical against several orthopoxviruses.

In recent studies, 4(3H)-Quinazolinone derivatives have been reported to possess antimicrobial and antiallergic properties (Dinakaran et al., 2003). In a cell culture study, Dinakaran et al. (2003) observed 6-Bromo-2-phenyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone exhibiting antiviral activity against VACV in E_6SM cell culture at a concentration of 1.92 µg/mL.

5.2 Baculovirus.

The effects of antiviral agents and disinfectants on baculoviruses have chiefly focused on their use in the laboratory for sterilizing insect diet preparations, or decontaminating work areas for the prevention of virus transmission during experiments (Jaques, 1977; Vail et al., 1968). Earlier studies demonstrated antiviral activity from formaldehyde incorporated into diets (Jaques, 1977; Ignoffo and Garcia, 1968; Vail et al., 1968). A study by Ignoffo and Garcia (1968) in which 0.04% formalin was added to suspensions of HzSNPV and TnNPV or incorporated into insect diets containing viruses, demonstrated the chemical agent reduces viral activity. Comparably, formalin solution (10%) has also been used to surface-sterilize eggs to prevent baculovirus infections (Paschke, 1964). In line with these findings, sodium hypochlorite (10%) has been used effectively as an egg surface sterilant (Ignoffo, 1963; Vail et al., 1968). Ignoffo and Dutky (1963) reported treatments of 0.5%, and 0.05% sodium hypochlorite for 1-30 min completely inactivated TnNPV. In 2001, Shapiro (2001) reported the cations strontium, ferrous and ferric chloride were inhibitory to virus activity of the *Lymantria dispar* Nuclear Polyhedrosis Virus (LdNPV).

Several reports have identified virucidal chemicals operating by denaturing the polyhedral protein matrix of viruses. In early studies, Andrews and Sikorowski (1973) reported significant swelling of HzSNPV polyhedra after exposure to 0.005M Na_2CO_3; based on these findings, it was concluded viral polyhedra would dissolve over time. Consistent with these observations, the inclusion body protein of insects was reported to dissolve several minutes after being exposed to 0.01M Na_2CO_3 (Jaques, 1977). Shapiro and Ignoffo (1969) reported carbonate dissolution of inclusion bodies from HzSNPV deactivated 99.9% of the virus. Furthermore, magnesium (0.003 M $MgSO_4$) reduced infectivity by 5-6 fold and a 7-fold decrease of infectivity was reported for β–mercaptoethanol (8 M; pH 7.6) (Shapiro and Ignoffo, 1969).

6. EFFECT OF ADJUVANTS, ADDITIVES, AND MICROENCAPSULATION ON BACULOVIRUSES

Formulation development is an important aspect for maximizing viral persistence characteristics of baculoviruses. Studies on the potential use of additives to

prolong viral simulant persistence in the field is particularly pertinent to a simulant decon program. Formulation research presented here addresses two central topics: (1) UV protection, and (2) storage stability: There is considerable evidence that Baculoviruses are inactivated soon after application to foliage exposed to direct sunlight (Arthurs and Lacey, 2004; Bullock, 1970; Ignoffo, 1992; Jaques, 1985). Efforts to stabilize baculoviruses against UV inactivation resulted in many sunscreening agents and microencapsulation procedures inhibiting loss of viral activity (Ignoffo et al., 1972; Ignoffo et al., 1976; Lacey and Shapiro-Ilan, 2003; Tamez-Guerra et al., 2000). Some account has already been given above on the protective nature of impure virus preparations. Agents added to the spray tank, separate from the biological pesticide formulation, to improve virus performance are known as adjuvants. Although several types of UV-protectants and adjuvants have been tested (dyes, reflectors, and absorbers), none have provided complete protection against sunlight; Baculoviruses, stabilized with UV-protectants generally break down at a slower rate.

6.1 Carbon.

Carbon products have received much attention as UV protectants (Ignoffo and Batzer, 1971; Ignoffo et al., 1972; 1997). In early studies, Ignoffo et al. (1972) observed virus persisted longer in the field stabilized with either carbon or IMC-90001, a natural polyflavinoid. The study demonstrated that activated carbon provided better UV protection than IMC-90001 (Ignoffo et al., 1972). Ignoffo et al. (1973) observed the residual activity of carbon-stabilized virus on corn silks was twice that of non-protected virus with virus activity recorded up to 24 days. Ignoffo et al. (1997) reported carbon and Benzopurpurin (a disazo dye) provided virus protection up to 48 hr from UV light. In the field, carbon provided the greatest protection; virus stabilized with other UV protectants significantly inactivated within 5-10 hr (Ignoffo et al., 1997). Formulations comprised of HzSNPV, talc and either activated carbon or Shade demonstrated good persistence against 24-hr exposure to simulated sunlight; >80% virus remained active (Ignoffo and Garcia, 1996). Ignoffo and Batzer (1971) reported that the encapsulation of HzSNPV with carbon black produced formulations remarkably resistant to inactivation by UV radiation with 87% of the virus still active after 4 hr exposure. Similar results with carbon black encapsulated formulations were reported by Bull et al. (1976) with over 50% of HzSNPV remained active following 48 hr exposure to UV radiation.

6.2 Cornstarch Products.

Generally granular formulations of HzSNPV based on cornstarch and solar protectants have demonstrated greater residual activity than unformulated viruses (Ignoffo et al., 1991). Formulations of HzSNPV containing a UV protectant are capable of prolonging inactivation between 2–6 fold (Ignoffo et al., 2001). Starch-encapsulated HzSNPV with the use of UV protectant, either Congo-Red dye or high-porosity-activated carbon, demonstrated improved virus persistence (Ignoffo et al., 1991). Tamez-Guerra et al. (2000) reported formulations of pregelatinized corn flour and potassium lignate provided protection against simulated rain (5 cm) and 8 hr of UV. The study also

reported a shelf-life of one year at ambient room temperature (Tamez-Guerra et al., 2000).

6.3 Enzymes.

One study by Ignoffo and Garcia (1994) investigated the effects of antioxidant and oxidative enzymes on UV inactivation by inhibiting the generation of highly reactive free radicals within HzSNPV. Water suspensions of inclusion bodies and protectant were exposed to simulated sunlight using UV-A (320-400 nm) and UV-B (280-320 nm) for 24 hr and 22 °C. All antioxidants tested provided some degree of viral protection; propyl gallate (0.2 mg/mL) was the most protective with 99% virus still active. Similar protection was found with phenylthiocarbamide and ascorbic acid at concentrations of 1.4 mg/mL and 38.0 mg/mL, respectively. At a concentration of 100 mg/mL, all oxidative enzymes provided protection with >87% virus still active. The concentration required to provide 50% protection with either catalase, peroxidase, or superoxide dismutase was 0.4 mg/mL, 3.8 mg/mL, or 2.3 mg/mL, respectively.

6.4 Lignin.

Studies have demonstrated adjuvants providing UV protection have significantly improved the activity of baculoviruses and prolonged virus viability (Arthurs et al., 2006; Ignoffo et al., 1976; Lacey and Shapiro-Ilan, 2003). Lignin is an abundant polymer derived from vascular plants and has been investigated as an adjuvant for microbial insecticides (Arthurs et al., 2006). One property of lignin is the ability to absorb visible wavelengths of light, which has made it a prime candidate for a UV protectant. Arthurs et al. (2006) reported spray-dried lignin-encapsulated CpGV formulations provided extended UV protection applied at a relatively high (3×10^{10} OB/L), but not low (3×10^{8} OB/L) virus dosages. In a similar study, McGuire et al. (2001) demonstrated formulations of *Anagrapha falcifera* Nuclear Polyhedrosis Virus (AfMNPV) containing lignin retained virus activity significantly longer than formulations without lignin. The presence of lignin in spray-dried virus formulations is also reported to improve baculovirus shelf-life. A study by Behle et al. (2003) demonstrated that lignin-based spray-dried formulations of AfMNPV had a shelf-life up to 3 months at 30°C; and as the temperature lowered to 4°C, the shelf-life increased to 30 months. In a study by Tamez-Guerra et al. (2002), 12 lignin-based formulations of AfMNPV were tested to determine storage stability. The experimental formulations were freeze-dried, stored either at room temperature (22°C) or 4°C, then tested at 6, 9 and, 12 months. Overall, results were inconsistent and provided no clear trend. Formulations containing sugar maintained viral activity better during storage than formulations lacking sugar; however, refrigerated virus generally remained more active when compared with un-refrigerated regardless of formulation.

6.5 Miscellaneous.

In a comprehensive laboratory test, Jaques (1971) evaluated 29 materials or combinations of solar protectants in suspension tests using purified TnNPV PIBs. After 40- or 6-min exposures to a germicidal lamp (254 nm), virus suspended in water containing an additive, either India ink, charcoal, brewer's yeast, yeast extract, peptonized milk, I.M.C. protectant, soy hydrolyzate, brilliant yellow stain, red ink, or autoclaved crude suspension of virus-killed insects retained at least 75% of original virus activity. Yeast extract, India ink, I.M.C. protectant and India ink + Plyac provided the greatest UV protection with at least 90% of original virus activity remaining after 60 min. In an initial field trial, Jaques (1971) reported the combination of India ink + egg albumen was the most promising, with deposits retaining at least 95% of their original activity 17 days after application. Furthermore, India ink (5%) retained 100% of the original virus activity after 5 days with >50% of original virus activity remaining after 17 days post application.

6.6 Titanium Dioxide.

Bull et al. (1976) observed virus encapsulations made with Titanium dioxide (TiO_2) helps prevent virus inactivation when exposed to UV radiation. TiO_2 prevents UV degradation by reflecting light energy rather than absorbing and is commonly used in paints, foods and sunscreens (McGuire et al., 2001). After 48 hr of exposure to a UV germicidal lamp, 25% of HzSNPV remained active (Bull et al., 1976). This finding agrees with Behle et al. (2003), who reported field experiments with TiO_2 added to lignin-based formulations extended viral persistence.

6.7 Stillbene-Disulfonic Acid Optical Brighteners.

Optical brighteners, which are stilbene-disulfonic acid derived, have demonstrated improved virus persistence in NPVs by acting as UV protectants (Boughton et al., 2001; Goulson et al., 2003; Li and Otvos, 1999; Martinez et al., 2004). Optical brighteners are commonly used in soaps, detergents and bleaches (Shapiro, 1992). The brighteners absorb light in the non-visible UV and violet portion of the spectrum and then re-emit visible light in the blue part of the spectrum (Boughton et al., 2001). Shapiro (1992) studied optical brighteners as UV protectants for formulations of the LdNPV and reported the best protection from the stilbene products with 100% original virus activity remaining after 14 days. Alternatively, in a study conducted by Vail et al. (1999), the stilbene-disulfonic acid derived optical brightener Tinopal LPW (M2R) was degraded by UV light within a few days. In support of these findings, Boughton et al. (2001) reported no UV protection from the optical brightener M2R.

6.8 Protectant/Virus Ratios.

There exists a paucity of research investigating the effect of ratio of virus to solid-protectant. Tamez-Guerra et al. (2000) reported formulations with a 2:1 ratio of solids to technical virus provided better solar and wash-off protection than formulations

with a 3:1 solids/virus ratio. In a UV protection study on encapsulated virus, Bull et al. (1976) observed promising results in the laboratory using formulations based on a mixture of titanium dioxide and carbon; however, these results were not reflected in field trials. Bull et al. (1976) concluded the solids/virus ratio could be adjusted to enhance UV protection. In contrast, Tamez-Guerra et al. (2002) reported inconsistent results from an experiment comparing the effects of formulation ratio (lignin and corn flour) to virus concentration on storage stability. No apparent trend resulted from the study; Tamez-Guerra concluded formulation composition was more important then ratio.

7. DISCUSSION AND CONCLUSIONS

Similarity with the poxvirus group is an important consideration in the practical use of baculoviruses for viral simulant Decon. This literature review compared and assessed the current state of understanding of the relative viability of vaccinia (poxviruses), and baculoviruses under different influencing factors and environmental conditions. The review identifies several physical properties of the baculovirus simulant candidates that correlate to the threat agent virus group while in turn solidifying baculoviruses as prime surrogates for viral simulant Decon.

Overall, the research focus on hardiness and persistence has been quite divergent for VACV and baculoviruses. Studies on VACV have traditionally focused on virus viability and potency of stored vaccine preparations, while baculovirus research is agriculturally based with a centralized focus on the improvement of formulation and insecticide performance (Falcon, 1969; Kaplan, 1958; Roberts and Hope, 2003). These divergent research topics and wide variety of experimental conditions have resulted in many datasets that are difficult to compare with one another. However, similar concepts on virus persistence and viability can be expected and several conclusions are drawn from these various conditions. Discussed below are 6 major physical and biochemical commonalities shared between VACV and baculoviruses. These similar characteristics generally demonstrate members of the baculovirus group as ideal simulant decon candidates.

(1) In general, pox and baculoviruses are viable under similar pH ranges. Near pH 7, VACV and baculoviruses remain stable, and from pH 4 to 8, these viruses show good short-term persistence (Andrews and Sikorowski, 1973; Ignoffo and Garcia, 1966; Rheinbaben et al., 2006). However, VACV and baculoviruses rapidly inactivate below pH 2 or above pH 11. Significant reduction in virus activity was reported for HzSNPV at pH 1.2 (Ignoffo and Garcia, 1966) with 88% virus inactivation after 24 hr of exposure at pH 2 (Gudauskas and Canerday, 1968); and immediate inactivation of VACV observed at pH 2.5 (Beard et al., 1938). Strong alkaline pH (>10) conditions are documented to dissolve the inclusion body protein in baculoviruses and inactivate the viruses (Kawarakata et al., 1980; Shapiro and Ignoffo, 1969). Pox viruses are reported less sensitive to extreme pH ranges due to low lipid content (Rheinbaben et al., 2006); however, like baculoviruses, exposure to alkaline ranges above pH 11, results in rapid virus inactivation (Beard et al., 1938).

(2) Based on a paucity of research, the most constructive generalization is to state VACV and baculoviruses are susceptible to inactivation under similar conditions of high moisture and RH. A greater loss of VACV viability was observed in aerosols at 80% RH when compared to lower RH conditions <50% (Harper, 1961). Similar results have been reported by Sidwell et al. (1966). Furthermore, McDevitt et al. (2007) demonstrated the survival of aerosolized VACV in the presence of UVC is significantly influenced by relative humidity. As the moisture content (RH) increases, VACV becomes more susceptible to UVC inactivation. Comparably, under conditions with relatively high moisture content, baculoviruses showed increased susceptibility to UV radiation. David (1969) observed PbGV inactivated more rapidly in wet virus films exposed to UV radiation than in dry films, as reported for TnNPV (Jaques, 1967) and, for HzSNPV (Ignoffo and Garcia,1992). Overall, studies have demonstrated under dry environs, VACV and baculoviruses respond similarly and remain relatively stable.

(3) Thermal sensitivities have been similar for VACV and baculoviruses. As discussed earlier, viral persistence may be affected by elevated temperatures through several mechanisms, which include protein denaturation, RNA damage, and influence on enzymatic activity or DNA replication. The effects of temperature on the persistence of pox and baculovirus groups have been examined in liquid and dried forms under a number of different conditions. Through these studies, a common trend arose for both virus groups; as expected, virus inactivation rates increased with increasing temperature. Temperatures exceeding 60 °C result in a rapid rate of virus inactivation. Virus inactivation curves produced by Kaplan (1968) and Woodroofe (1960) demonstrated a significant loss of VACV activity at temperature ranges between 50-60 °C. In a liquid medium (CAM suspension), VACV completely inactivated at 60 °C after 2 hr; at 70 °C after 30 min; and at temperature ranges of 80 °C and above after 10 min (Shchelkunov et al., 2005). Though thermal stability studies have produced diverse results, baculoviruses, like poxviruses, are inactivated by relatively short exposures to high temperatures. Studies have demonstrated that baculoviruses were completely inactivated by exposure as suspensions or dried to temperatures of 70 to 80 °C for 10 min (Bedford, 1981; Jaques, 1977; Martignoni and Iwai, 1977). Conversely, research has shown lower temperatures (<5 °C) generally enhance virus survival and most viruses can be stored frozen to maintain their infectivity for long periods of time. Studies have documented VACV still viable after 15 years stored at -20 °C (Rheinbaben, 2007); comparably, HzSNPV remained active after 25 years stored at 5 °C (Ignoffo, 1992). The slight differences in the effects of temperatures between the virus groups (e.g., pox and baculovirus) may be due to differences of the virus type, the temperatures to which they are stored/frozen, the rate of freezing and thawing and the medium in which they are stored/frozen.

(4) Exposure to formalin and sodium hypochlorite inactivated both VACV and a large proportion of baculoviruses (Grossgebauer et al., 1975; Jaques, 1977; Vail et al., 1968). Formaldehyde vapor, as well as in suspension, completely inactivated VACV (Grossgebauer et al., 1975; Schümann and Grossgebauer, 1977). In line with these findings, formaldehyde has been successfully used as surface disinfectants for

insect diets against baculovirus contamination (Paschke, 1964). Furthermore, in suspension tests with HzSNPV and TnNPV, formaldehyde inactivated viruses (Garcia, 1968). Comparably, sodium hypochlorite has been used effectively as a disinfectant of baculoviruses and VACV for work areas in laboratories, as well as Healthcare and food industry facilities (Vail et al., 1968).

(5) In general, studies have demonstrated that pox and baculoviruses are readily inactivated by UV radiation. The majority of studies on UV radiation inactivation of VACV have focused primarily on germicidal UV cell light (254 nm) for the control of microbial infection spread; exposure to this wavelength results in rapid inactivation of VACV (<10 s). Similarly, Gudauskas and Canerday (1968) documented suspensions of HzSNPV and TnNPV rapidly inactivated when exposed to these wavelengths with a complete loss of activity in <10 min. Bullock et al. (1970) reported similar findings at 2-hr exposure to wavelengths of 257 nm. These studies suggest common physical characteristics shared between the pox and baculoviruses; exposed to a germicidal UV wavelength (254 nm), both virus groups are quickly inactivated. Furthermore, reports from Sime and Bedson (1973) and Rauth (1965) suggest VACV is susceptible to UV ranges between 280-300 nm. However, a thorough search of literature revealed few studies to make direct comparisons at higher wavelengths. Additional work is required to identify and compare different UV wavelengths on the stability of pox and baculoviruses.

(6) Insect metabolism did not appreciably reduce VACV or baculovirus activity. Overall, the effect of natural biological activity on the survival of viruses is poorly documented. However, data show VACV and *Helicoverpa punctigera* NPV respond similarly when passed through insect digestive tracks. Results from these experiments have found no difference between virus inactivation rates (Bartzokas et al., 1978; Beckman, 1980); virus particles excreted up to 4-5 days after insect ingestion remained active. The prolonged persistence of these viruses attests to a relatively comparable effect of insect metabolism on viral inactivation.

Most importantly, the literature review identified several substances and treatment conditions, which make VACV susceptible to inactivation that in turn can be similarly tested on the baculovirus simulants. This presents an important step in decontamination science to solidly confirm baculoviruses as viral simulant substitutes for poxviruses. Virions from CpGV and HzSNPV can be subjected to these treatments following methods described in the parent study. The resulting experimental data can then be compared to published data, and side-by-side experiments can be designed and executed to validate findings. Final results can then be used to further determine and conclude on the potential of baculoviruses as components to a viral simulant research program.

The literature review on VACV and baculovirus viability identified the following virucidal compounds and conditions to test:

- Antiviral effects of Amphotericin B Methyl Ester by a plaque reduction assay (Jordan and Seet, 1978).

- Toxicity of glutaraldehyde and oxidized spermine using a plaque reduction assay (Bachrach and Rosenkovitch, 1972).

- Antiviral effects of 6-Bromo-2-phenyl-3-[(4-amino-5-(4-chlorophenyl)-6-ethylpyrimidin-2-yl]-4(3H)-quinazolinone in cell culture assay (Dinakaran et al., 2003).

- Virucidal effects of an alcohol-based formulation including 55% ethanol (w/w) with 10% (w/w) propan-1-ol, 5.9% (w/w) propan-1.2-diol, 5.7% (w/w) butan-1.3-diol and 0.75% phosphoric acid in a suspension test (Kramer et al., 2006).

- Toxicity of 0.5% monopercitric acid (MPCA) by suspension test (Wutzler and Sauerbrei, 2004).

- Virucidal effects of alcohol-based hand rubs by suspension test (Kampf et al., 2007).

- Evaluation of virucidal effects of three surface disinfectants (quaternary ammonium, phenolic, and iodophor) on a simulated hard environmental surface (Gaustad et al., 1974).

- Virus inactivation by solvent/detergent treatments of TNBP/Tween 80 or TNBP/Cholate in suspension tests (Remington et al., 2004).

- Virucidal effects of ascorbic acid by suspension test (Turner, 1964).

- Sensitivity of aerosolized virus at predetermined temperature and humidity ranges (Henderson et al., 1999).

- Sensitivity of aerosolized virus exposed to UVC (254 nm) under the influence of relative humidity (McDevitt et al., 2007).

- Determination of virus persistence on various surfaces including cotton and wool fabrics (Sidwell et al., 1966); and plant materials (Bullock, 1967).

In conclusion, the rate of inactivation and the extent of virus survival vary, depending upon virus type (pox or baculovirus), temperature, UV light exposure as well as other environmental and abiotic conditions. Overall, a lack of comparative studies exists between pox and baculoviruses, which stems from the employment of these virus groups for different purposes. In general, the baculoviruses, specifically CpGV and HzSNPV, have demonstrated an overall similarity to VACV. This report supports the adoption of baculoviruses as potential viral simulants for poxviruses in decontamination science. However, additional quantitative information is required for a direct comparison of VACV and baculoviruses on the survival, persistence and inactivation

rates in various environmental conditions such as wet states (as aerosols or under high RH), when subjected to drying and desiccation, when deposited on various surfaces (textiles, soil or plant materials), when aerosolized, when subjected to chemical treatments such as virucides, disinfectants and other germicidal treatment chemicals (e.g., aldehydes, alkaline material treatments), and when subjected to physical process such as, UV irradiation, and extreme changes in temperature.

Table 1. Effect of UV Radiation on Virus Viability

Virus	UV Wavelength (nm)	Exposure Time	Loss of Activity (Estimated %)	Conditions	References
Helicoverpa spp. NPV	257	2 hr	>50	1-phenyl-2-thiorea suspension; 24 - 26 °C	Bullock et al., 1970
Helicoverpa spp. NPV	307.5	2 hr	>50	1-phenyl-2-thiorea suspension; 24 - 26 °C	Bullock et al., 1970
Helicoverpa spp. NPV	364	2 hr	<10	1-phenyl-2-thiorea suspension; 24 - 26 °C	Bullock et al., 1970
HzSNPV	254	5 min	100	Suspension; petridish. 2 in. from light	Gudauskas and Canerday, 1968
HzSNPV	(215-260) and (290-400)	4 hr	62	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	(215-260) and (290-400)	12 hr	67	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	(215-260) and (290-400)	24 hr	73	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	(215-260) and (290-400)	48 hr	80	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	(215-260) and (290-400)	96 hr	87	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	(215-260) and (290-400)	192 hr	90	Dry film on glass petridish; 25 °C	Ignoffo and Batzer, 1971
HzSNPV	254 and 365	4 hr	90	Dry film on glass petridish	Ignoffo et al., 1977
LfNPV	366	100 hr	<10	Suspension; open petridish	Morris, 1971
OpNPV	290	-	32.7 - 53.5	Water Suspension; Room Temperature;	Griego et al., 1985
OpNPV	300	-	0 – 74.2	Water Suspension; Room Temperature	Griego et al., 1985
OpNPV	310	-	0 - 82	Water Suspension; Room Temperature	Griego et al., 1985
OpNPV	320	-	0 - 51.3	Water Suspension; Room Temperature	Griego et al., 1985
PrGV	254 and 365	4 hr	95	Dry film on glass petridish	Ignoffo et al., 1977
VAC	254	0.3 - 0.6 s	99.9	Eagles Medium suspension; RH = 65%	Jensen, 1964
VAC	254	7.6 s	99*	Water; RH = 18-23%; Dose = 2 J/m^2	McDevitt et al., 2007
VAC	254	7.6 s	90*	Water; RH = 56-63%; Dose = 1.5 J/m^2	McDevitt et al., 2007
VAC	254	7.6 s	90*	Water; RH = 78-83%; Dose = 1.75 J/m^2	McDevitt et al., 2007
VAC	254	7.6 s	90*	SRF; RH = 18-23%; Dose = 3.25 J/m^2	McDevitt et al., 2007
VAC	254	7.6 s	90*	SRF; RH = 56-63%; Dose = 1.75 J/m^2	McDevitt et al., 2007

Table 1. Effect of UV Radiation on Virus Viability (Continued)

Virus	UV Wavelength (nm)	Exposure Time	Loss of Activity (Estimated %)	Conditions	References
VAC	254	7.6 s	80*	SRF; RH = 78-83%; Dose = 2.75 J/m^2	McDevitt et al., 2007
VAC	200-1100 (PureBright)	300 µs	100	PureBright® system; Dose = >1.5 J/cm^2	Roberts and Hope, 2003

* Study presented dose response curves, here we report only on 90 or 99% inactivation of virus.

Table 2. Effect of Storage Temperatures on Virus Stability

Virus	Temperature (°C)	Exposure Time	Loss of Activity (Estimated %)	Condition	References
HzSNPV	75	10 min	ca. 40	10 mL suspension	Gudauskas and Cannerday, 1968
HzSNPV	80	10 min	100	10 mL suspension	Gudauskas and Cannerday, 1968
HzSNPV	88	10 min	100	10 mL suspension	Gudauskas and Cannerday, 1968
HzSNPV	5	25 years	still active	-	Ignoffo, 1992
HzSNPV	50	100 days	100	-	Ignoffo, 1992
HzSNPV	5	30 days	ca. 50	Carbon dissolution	Shapiro and Ignoffo, 1969
HzSNPV	37	60 days	ca. 50	Carbon dissolution	Shapiro and Ignoffo, 1969
HzSNPV	50	120 days	ca. 50	Carbon dissolution	Shapiro and Ignoffo, 1969
HzSNPV	37.7	2 hr	<10	7 mL suspension in di-H_2O	Stuermer and Bullock, 1968
HzSNPV	60	2 hr	<10	7 mL suspension in di-H_2O	Stuermer and Bullock, 1968
HzSNPV	71.1	15 min	>50	7 mL suspension in di-H_2O	Stuermer and Bullock, 1968
HzSNPV	82.2	2 hr	>95	7 mL suspension in di-H_2O	Stuermer and Bullock, 1968
HzSNPV	93.3	15 min	>95	7 mL suspension in di-H_2O	Stuermer and Bullock, 1968
PbGV	20-22	2 days	~50	Dried film on glass; RH = 60%	David et al., 1971b
PbGV	20-22	<1 hr	10-15	Dried film on glass; RH = 60%	David et al., 1971b
PbGV	20-22	7 days	>80	Dried film on glass; RH = 60%	David et al., 1971b
PbGV	20-22	14 days	>95	Dried film on glass; RH = 60%	David et al., 1971b
TnNPV	80	10 min	ca. 70	10 mL suspension	Gudauskas and Cannerday, 1968
TnNPV	82	10 min	ca. 97	10 mL suspension	Gudauskas and Cannerday, 1968
TnNPV	88	10 min	100	10 mL suspension	Gudauskas and Cannerday, 1968
VAC	4	245 days	0	Dried form	Rheinbaben, 2007
VAC	-20	15 years	-	Frozen in Buffer	Rheinbaben, 2007
VAC	50	15 hr	100	Suspension in McIlvaine's buff.	Woodroofe, 1960
VAC	55	2.5 hr	100	Suspension in McIlvaine's buff.	Woodroofe, 1960
VAC	60	15 min	100	Suspension in McIlvaine's buff.	Woodroofe, 1960

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GLOSSARY

AfMNPV	*Anagrapha falcifera* Nuclear Polyhedrosis Virus
AgNPV	*Anticarsa gemmatalis* Nuclear Polyhedrosis Virus
BmNPV	*Bombyx mori* Nuclear Polyhedrosis Virus
CpGV	*Cydia pomonella* Granulovirus
GV	Granulovirus
HzSNPV	*Helicoverpa zea* Nuclear Polyhedrosis Virus
IPM	Integrated Pest Management
LdNPV	*Lymantria dispar* Nuclear Polyhedrosis Virus
LfNPV	*Lambdina fiscellaria* Nuclear Polyhedrosis Virus
NPV	Nuclear Polyhedrosis Virus
NsNPV	*Neodiprion sertifer* Nuclear Polyhedrosis Virus
OB	Occlusion bodies
OpNPV	*Orgyia pseudotsugata* Nuclear Polyhedrosis Virus
PAA	Paracetic acid
PbGV	*Pieris brassicae* Granulovirus
PIB	Polyhedral inclusion bodies
PrGV	*Pieris rapae* Granulovirus
TNBP	Tri-(n-butyl)-phosphate
TnGV	*Trichoplusia ni* Granulovirus
TnNPV	*Trichoplusia ni* Nuclear Polyhedrosis Virus
VACV	Vaccinia virus
VARV	*Variola virus*
WHO	World Health Organization